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RESPIRATORY STUDIES OF SINGLE CELLS.¹ III. OXYGEN CONSUMPTION DURING CELL DIVISION

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The reference diver technique (Scholander, Claff and Sveinsson, 1952a) has made it possible to study in detail the oxygen consumption of large single cells. In a previous paper (Scholander, Claff and Sveinsson, 1952b) we reported observations on single protozoans, and in the present study we present data on the oxygen consumption during the first four cell divisions of the fertilized eggs of two sea urchins, *Strongylocentrotus purpuratus* and *S. franciscanus*, a sand dollar, *Den- draster excentricus*, and the echiuroid worm, *Urechis caupo*.

During mitosis a series of events takes place, all of which require energy in one form or another. These events could conceivably occur within the limits of a perfectly steady total flow of energy expenditure or they might present themselves as bumps or cycles on top of a "basal" energy exchange. The gross process of cleaving one lump of protoplasm into two or more pieces is one phase which could readily be expected to be associated with such an excess oxygen intake. In aerobic eggs the energy necessary for mitosis may in the last analysis be oxidative, and it is therefore natural to look for cycles in the oxygen consumption correlated to the anatomical events. Many authors have looked for such cycles in the oxygen consumption with both positive and negative results. For a complete survey the reader is referred to the excellent monographs by Needham (1931) and Brachet (1950).

Gray (1925) in two mass run measurements on *Echinus esculentus*, using a Barcroft respirometer, found no cycling in the oxygen consumption. By re-calculating and plotting his data according to the procedure which is described below, it is apparent that in one of his runs there are actually very slight accelerations in the rate associated with each of the three cleavages.

Runnström (1933a), using the Warburg apparatus, in mass runs with *Paracentrotus lividus*, found an increase in oxygen consumption starting at the appearance of the cleavage furrow. Brachet (1932, 1934, 1935, 1950), using in most experiments a Fenn respirometer, found cycling in the oxygen consumption of frog eggs (*Rana*

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fusca). In 34 runs with some 80 eggs per respirometer, he found that the peaks of his curves coincided with the appearances of the cleavage furrow.

Zeuthen (1949, 1950a, 1950b, 1950c) found cycling to be of constant occurrence in eggs of *Psammechinus miliaris*, *Strongylocentrotus franciscanus*, *Dendraster excentricus* and *Urechis caupo*, which were run several hundred at a time in modified Cartesian divers. The cleavage usually took place when the rate of oxygen consumption was at a minimum. In all of the echinoderms mentioned he found that the cycling increased markedly in intensity after the fifth to seventh division. Tang (1948) reports similar results on *Arbacia punctulata*, using the Warburg technique.

In all mass runs one must depend upon the degree of synchronism of the cleavages of a great number of eggs. There is necessarily some individual variability in the cleavage rhythm, and one faces the risk of promoting asynchronism by tension gradients developing in a sedimented cell mass. Whatever the reasons may be for differences in the individual rhythm of eggs, it would seem likely that the cell divisions would get progressively more out of step at each successive division. As amply demonstrated, especially by Zeuthen, the statistical approach has yielded extremely valuable information. A certain degree of blurring of the details is inevitable in mass determinations, however, and it is hence of interest whenever possible to supplement the information by runs on single eggs. This has hitherto been possible only for very large eggs, such as that of the frog. Zeuthen (1946) studied single frog eggs (*Rana platyrrhina*) in modified Cartesian divers, and found that cycling was a regular feature, with cleavage beginning at or slightly after a respiratory minimum. Cycling was also found to take place without cytoplasmic division.

In the present investigation we have undertaken to analyze on single eggs the oxygen consumption during cell division in several marine species. With the exception of *S. purpuratus*, Zeuthen (1949, 1950c) has studied the same forms in mass runs.

MATERIAL AND METHODS

The echinoderm material was secured at Friday Harbor, Washington, and *Urechis* at Pacific Grove, California.

Eggs and sperm from *S. purpuratus* and *Dendraster* were obtained either by spontaneous spawning or by injection of isotonic KCl solution. The spawning season was over for *S. franciscanus*, but enough eggs for our purpose were obtained by removing the ovary and shaking pieces of it in cold sea water, washing out a dozen eggs or more. The eggs were then rinsed free of all debris in several dishes of sterile and cool sea water. All dishes were kept cool.

Dendraster has a thick and colorful "furry" coating on the egg. This was stripped off from the fertilization membrane by passing the egg repeatedly through a narrow pipette opening.

The eggs and sperm of *Urechis* were taken by pipette from the genital pores. In this species all runs were performed on material from the same two animals, whereas in the echinoderms new pairs were used each time. Much difficulty was encountered with the *Urechis* eggs in the beginning because the egg adhered badly to the chamber wall and made the diver stick. It required many passings of the egg through a pipette to get rid of this stickiness.

At zero time the eggs were fertilized by stirring them with a fine glass tip which

had been touched to a cold and concentrated sperm suspension. The fertilization was followed under a dissecting microscope and as soon as the membrane had developed, a perfect-looking egg was picked for the run. This egg was transferred through two or three dishes of cold sterile sea water and loaded into the chamber, which was kept cold by having the supporting stopper chilled beforehand. The centrifuge was kept in the refrigerator. Sterile technique was used throughout (see Scholander *et al.*, 1952a).

PRESENTATION OF DATA

In Figure 1 the cumulative oxygen consumption of the *Dendraster* runs has been plotted in $\mu\mu\text{l}$, omitting most readings that are less than three minutes apart. Under each of these curves we have drawn a straight line representing an average rate measured in $\mu\mu\text{l}/\text{min}$. We are particularly interested in seeing to what degree the observed curves deviate from the straight lines. To find this we calculated a series of figures for the average oxygen consumption corresponding in time to the observed

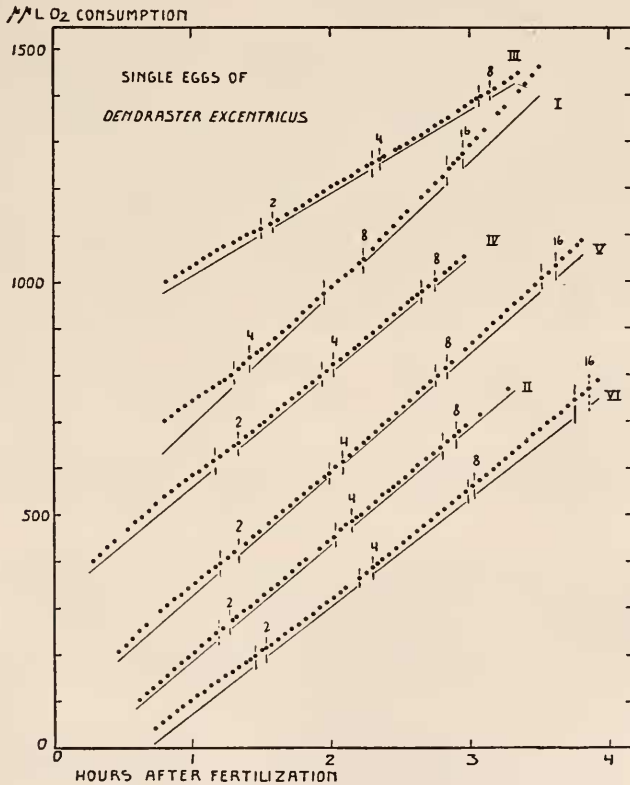


FIGURE 1. Cumulative total oxygen consumption during cell divisions in single eggs of the sand dollar, *Dendraster excentricus*. The beginning and end of each cell division are marked by vertical lines and the number of cells at the end of the division is indicated. Under each curve is drawn a straight line taken as the average rate of consumption. The position of each curve on the ordinate scale is arbitrary.

figures. The two sets of figures were subtracted from each other point by point, giving the absolute deviations from linearity in μl . The deviations were plotted at their proper times and could now be sufficiently enlarged to permit a detailed analysis. The curve thus obtained is slightly skewed to the left because the values are plotted at an angle from the original ordinates. This effect is entirely negligible when the oscillations are as small as those presented. We have chosen this way of treating the data rather than recalculating the observations as a change of rate from point to point. The latter procedure has been most commonly used but is wasteful of good data if the observation points are very close together. The present curves represent simply the deviations from a straight line re-plotted on a magnified scale.

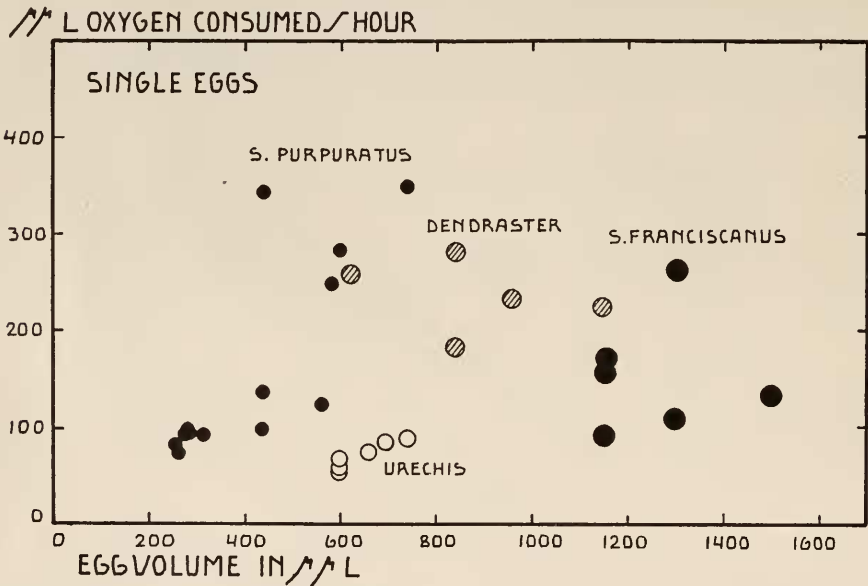


FIGURE 2. Cell volume in relation to rate of oxygen consumption in single marine eggs through the first three to four cell divisions after fertilization. Each point represents data from one egg. Tp. 15° - 17° .

The results of the oxygen consumption determinations in four species of marine eggs are given in Figures 3-6. At each division the first appearance of a cleavage furrow is marked with one vertical line, and the complete separation of the cells with a second line. Up to the eight cell stage these lines are quite accurate, as all the cells could be clearly seen by turning the chamber. The beginning of the cleavage at the 8-16 cell division is accurate to about one minute, whereas the end, when the last cell finished dividing, is less certain. It is quite difficult to determine accurately the starting and finishing times for the 16-32 cell division, and for the next division (32-64 cells) they are even more difficult to see.

In Figures 3-6 the rate is represented by the slope of the curves. At the average rate they run horizontal. Above the second division a point has been located which corresponds to a 10% increase in the oxygen consumption in the period

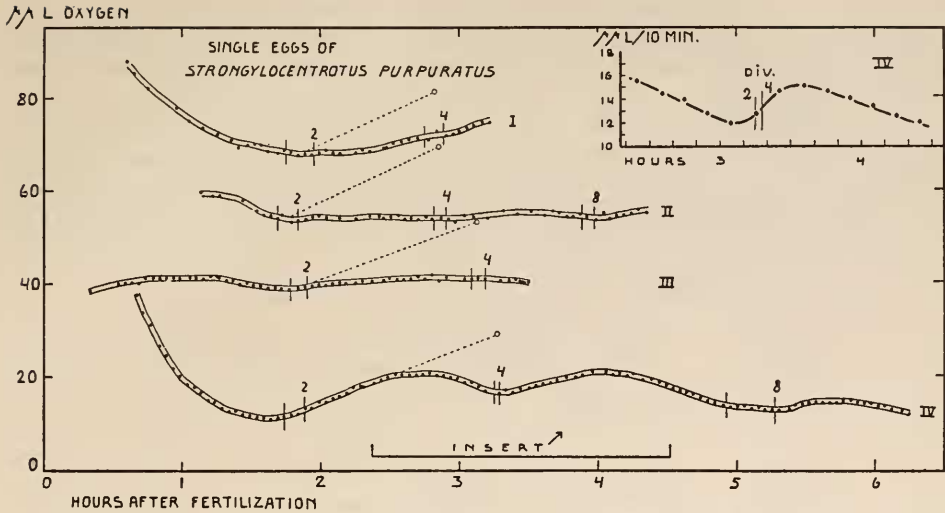


FIGURE 3. Deviations from a linear rate of oxygen consumption during cell division in single eggs of a sea urchin, *Strongylocentrotus purpuratus*. The average rate runs horizontal. A 10% increase of rate is indicated by the slope of the dotted lines. The ordinate units represent the absolute deviations given in $\mu\mu\text{l}$. The parallel lines indicate the spread of the observations and are drawn $1 \mu\mu\text{l}$ apart. The beginning and end of the cell cleavages are indicated by vertical lines and the digit at the end of the division gives the resulting number of cells. The position of each curve on the ordinate scale is arbitrary. Insert: Section of Curve IV recalculated as a rate curve.

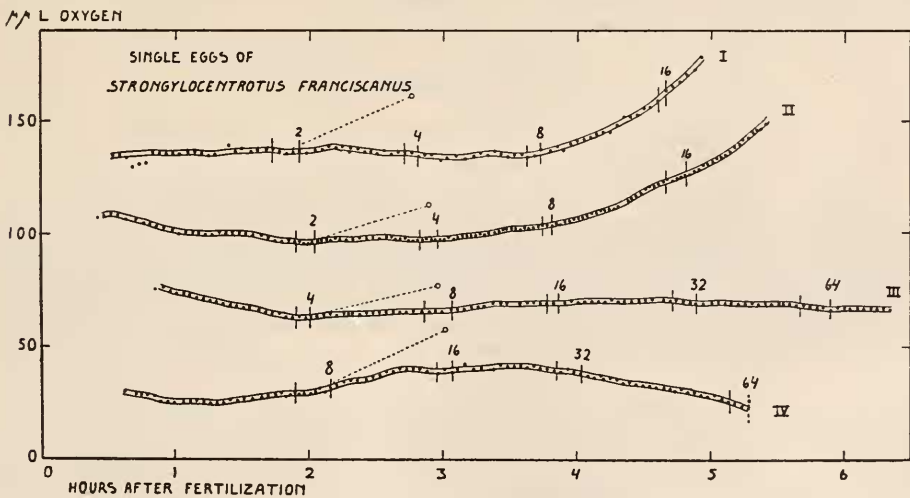


FIGURE 4. Deviations from a linear rate of oxygen consumption during cell division in single eggs of a sea urchin, *Strongylocentrotus franciscanus*. The average rate runs horizontal. A 10% increase of rate is indicated by the slope of the dotted lines. The ordinate units represent the absolute deviations given in $\mu\mu\text{l}$. The parallel lines indicate the spread of the observations and are drawn $2 \mu\mu\text{l}$ apart. The beginning and end of the cell cleavages are indicated by vertical lines and the digit at the end of the division gives the resulting number of cells. The position of each curve on the ordinate scale is arbitrary.

between the first two divisions, and the slope of a 10% rate change is given by the dotted line. The magnitudes of observed changes in rate can hence be estimated by comparing them with the slope of this line. The absolute deviations are directly plotted as ordinates.

Two parallel lines have been drawn one on each side of the observed points, encompassing their scattering. The distance between these lines and hence the scattering varies from $0.5 \mu\mu\text{l}$ to $2 \mu\mu\text{l}$. Some of the curves show a slow increase of rate throughout the experiment. We dare not attach significance to these slow

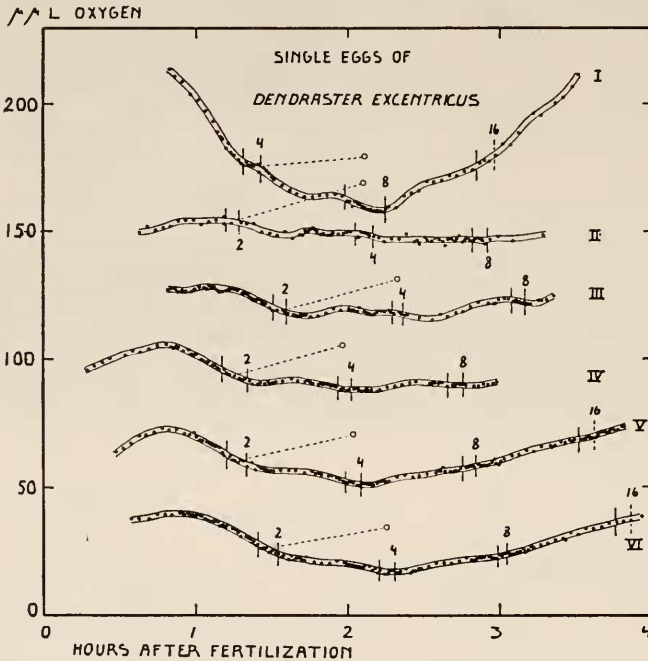


FIGURE 5. Deviations from a linear rate of oxygen consumption during cell division in single eggs of a sand dollar, *Dendraster excentricus*. The average rate runs horizontal. A 10% increase of rate is indicated by the slope of the dotted lines. The ordinate units represent the absolute deviations given in $\mu\mu\text{l}$. The parallel lines indicate the spread of the observations and are drawn $2 \mu\mu\text{l}$ apart. The beginning and end of the cell cleavages are indicated by vertical lines and the digit at the end of the division gives the resulting number of cells. The position of each curve on the ordinate scale is arbitrary.

trends as they are in magnitude inside the possible limits of a base line drift (Scholander *et al.*, 1952a, Fig. 7), and the possibility of infection cannot be entirely discounted. A slight steady drift does not, however, obscure the relatively short-time details in which we are interested.

RELATION OF EGG VOLUME TO OXYGEN CONSUMPTION

The egg cell diameter, not including the fertilization membrane, was measured, and from this the volume calculated, considering the cell a sphere. In *Urechis* the

measurement was taken after the caved-in pole had swelled out. In Figure 2 the egg volume is plotted against the average oxygen consumption during the first three to four cleavages. It will be seen that there is a very considerable variability in individual eggs as to both size and rate. In *S. purpuratus* the oxygen consumption varied two- to threefold for the same egg volume and there is on the whole poor or no correlation with size. *Paramecium* with the same range of size as these eggs had a metabolic rate about 5–10 times higher (Scholander *et al.*, 1952b).

Published data from mass runs for the most part fit well with our data. Tyler and Humason (1937) found an average O_2 consumption of *Dendroaster excentricus*

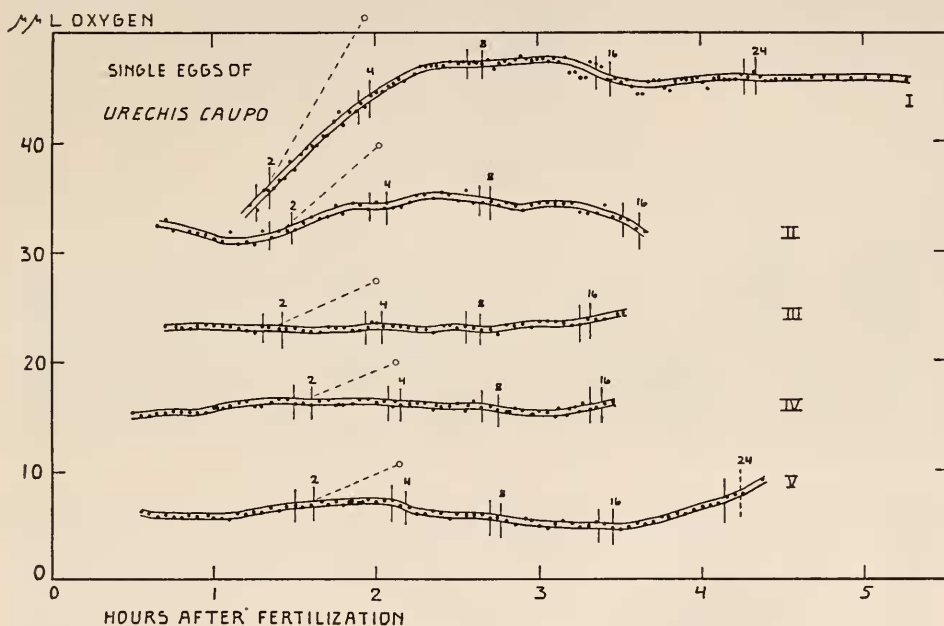


FIGURE 6. Deviations from a linear rate of oxygen consumption during cell division in single eggs of an echiuroid worm, *Urechis caupo*. The average rate runs horizontal. A 10% increase of rate is indicated by the slope of the dotted lines. The ordinate units represent the absolute deviations given in $\mu\mu\text{l}$. The parallel lines indicate the spread of the observations and are drawn $0.5 \mu\mu\text{l}$ apart. The beginning and end of the cell cleavages are indicated by vertical lines and the digit at the end of the division gives the resulting number of cells. The position of each curve on the ordinate scale is arbitrary.

of $208 \mu\mu\text{l}/\text{hour}$ at 15° . Lindahl and Holter (1940) gave an average egg volume of $584 \mu\mu\text{l}$ and an O_2 consumption (18°) of $155 \mu\mu\text{l}$ for *Paracentrotus lividus*. Borei (1948) obtained an average egg volume of $556 \mu\mu\text{l}$ for *Psammechinus miliaris*, with an O_2 consumption (18°) of $184 \mu\mu\text{l}$. He found *Asterias glacialis* to have a very large egg of $2520 \mu\mu\text{l}$ with an O_2 consumption (18°) of only $250 \mu\mu\text{l}$. *Arbacia punctulata*, with an egg volume of $190\text{--}260 \mu\mu\text{l}$ (Tang, 1931), has an oxygen consumption between 34 and $100 \mu\mu\text{l}$ (Krahl, 1950).

The oxygen consumption in our material of single fertilized eggs varies greatly

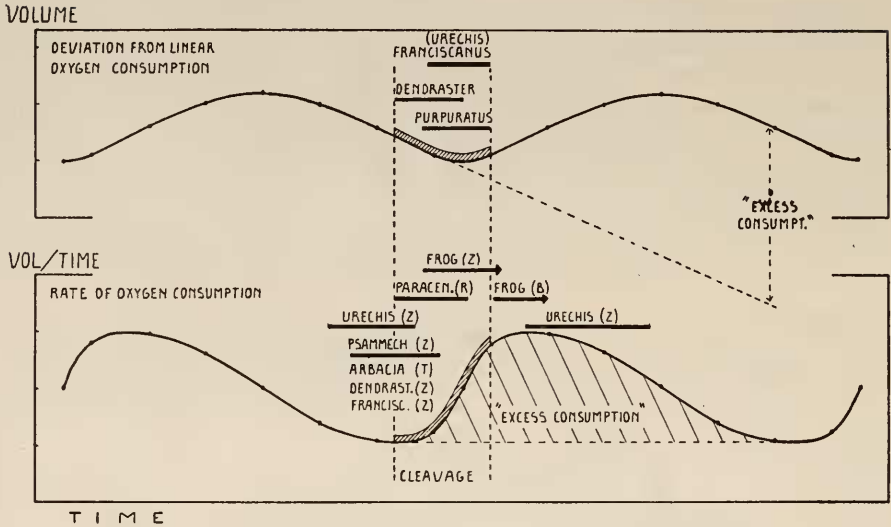


FIGURE 7. Generalized curves of cycling in the oxygen consumption of marine eggs. Upper: Curve showing the deviation from a linear rate of oxygen consumption such as obtained in the present investigation (Figs. 3-6). The region where cleavage occurred is given by horizontal lines. "Excess" oxygen consumption is indicated by a distance. Lower: This curve represents the upper curve recalculated as a conventional rate curve. The horizontal lines represent the regions where cytoplasmic cleavage started and ended, as found by different authors (B = Brachet, R = Runnström, T = Tang, Z = Zeuthen). The arrows (frog data) denote the region where the furrows started. "Excess" oxygen consumption is indicated by the shaded area.

individually with a poor or no correlation to size, and on the whole it seems doubtful that a near linear relation such as indicated by Smith and Kleiber (1950) exists.

RELATION OF OXYGEN CONSUMPTION TO CELL CLEAVAGE

The deviations from an average linear oxygen consumption have been plotted for fertilized eggs of four marine species (Figs. 3-6). Data pertaining to these runs and not given in the figures are presented in Table I.

Strongylocentrotus purpuratus. This species has very slowly cleaving eggs. Figure 3, curve IV shows a considerable cycling with total rate changes of about 20-25%. In this run the third cell division seemed delayed and showed poor synchronization, leaving one cell undivided. The cyclings decrease strongly in amplitude with time. If we interpret the increase of rate after, for example, the second division, as an "oxygen debt", it would, according to the construction given in Figure 7, amount to about 10-15% of the oxygen consumed during the period between the divisions. The rate increase by the cell division may well have other causes. In the insert on Figure 3 we have given the data before, during, and after the second cell division, recalculated from curve IV of Figure 3 as a conventional rate curve. The abrupt increase of rate at the cell division and the subsequent slow decline are clearly visible. We have not been able to find any clue from the data presented in Table I as to why this egg, IV, showed such a strong cycling.

Curve III shows an increase of rate at the first cell division of about 8%, suggestive of cycling. Curve II shows also an increase of rate at the cell divisions. Curve I exhibits a steadily increasing oxygen consumption and is inconclusive as to cycling.

S. franciscanus. In Figure 4, curves II, III, and IV, there are slight rate increases at the first and second divisions suggestive of cycling, but later there are none. When the single cell in curve III divided directly into four cells there was about a 15% increase in rate, or about twice that seen in II where the egg cleaved only into two. In curve IV the single cell sprang directly into eight cells with no more increase, however, than the cell which cleaved into two. It would seem likely that splitting into eight would represent more of a disturbance than splitting into two, and that it would show up in the oxygen consumption if the eggs were otherwise the same. This discordance may be explained by the fact that different eggs may

TABLE I
Oxygen consumption in marine eggs
Data pertaining to Figures 3-6

No.	Temp.	$\frac{\text{mm. Hg}}{\text{min.}}$	$\frac{\text{Factor}}{\text{mm. Hg}}$ $\frac{\mu\mu\text{l O}_2}{\text{mm. Hg}}$	$\frac{\mu\mu\text{l O}_2}{\text{hour}}$	Egg vol. $\mu\mu\text{l}$	Water vol. $\mu\mu\text{l}$	$\frac{\text{Water vol.}}{\text{Egg vol.}}$	$\frac{\text{Water vol.}}{\text{Bubble vol.}}$ of 200 $\mu\mu\text{l}$
<i>Strongylocentrotus purpuratus</i>								
I	16°	0.9	1.8	98	280	40,200	144	201
II	16°	1.9	1.3	140	440	20,900	48	105
III	16°	2.7	0.6	98	280	11,800	42	59
IV	16°	1.6	0.9	83	255	18,700	74	94
<i>S. franciscanus</i>								
I	16°	1.4	3.4	267	1300	84,600	65	423
II	16°	2.0	1.4	165	1150	33,500	29	168
III	16°	1.9	1.0	111	1300	22,400	17	112
IV	16°	1.9	1.5	171		36,200		181
<i>Dendraster</i>								
I	15°	1.2	4.0	282	840	97,600	116	488
II	15°	1.2	3.4	252	600	81,500	136	408
III	15°	1.1	2.8	175		65,100		326
IV	15°	2.4	1.7	246	1150	37,800	33	189
V	15°	2.2	2.0	261		45,000		225
VI	15°	2.2	1.8	234	960	40,200	42	201
<i>Urechis</i>								
I	17°	1.0	1.5	90	740	33,600	45	168
II	17°	1.0	1.3	78	660	29,200	44	146
III	17°	1.1	1.1	69	600	26,200	44	131
IV	17°	0.9	1.1	61	600	26,200	44	131
V	17°	1.1	0.8	55	600	17,800	30	89

cycle to a very different degree (*cf.*, *S. purpuratus*). The later cell divisions in both runs, III and IV, were of normal appearance. At the end of these runs the cells were taken out of the chamber and placed in dishes, where they developed into perfectly normal-appearing plutei.

Dendroaster excentricus. This is the only egg tested that seems to cycle regularly. The cycling is strongly damped and almost vanishes after the third division (see Figure 5, V and VI). The cleavage takes place after a low rate and is immediately followed by a rise. In curves I, V, and VI the cycling is superimposed upon a general increase in the oxygen consumption, which gives the impression of a persisting, stepwise increase at each division. It cannot be definitely excluded that this general increase may have been caused by baseline drift or possibly infection. Most cleavages were recorded with observations every minute, in order to see if any relatively rapid changes would take place immediately at the cleavage. No such changes were found.

Urechis caupo. This animal has a slowly metabolizing egg and great care was taken to minimize the spread of the observations, which in the later runs (Figure 6, III, IV, and V) were within 0.5 $\mu\mu$ l. No cycling could be observed except in number II, where it appears rather clearly.

As a result of the above described runs on single eggs we may say that the cell division in many cases takes place without any visible change in rate of oxygen consumption. When a change does occur it is most commonly seen as a rather abrupt increase of rate at the first cell cleavage. Sometimes this increase subsides and is repeated at the next cleavage. We then get cycles in the oxygen consumption. These were clearly present only in *S. purpuratus* and *Dendroaster*, and of inconstant occurrence in both. The cycles have a shape usually like the one drawn in Figure 7 (upper), rather than a regular sine shape. This generalized curve has been recalculated into a rate curve (Fig. 7, lower). It will be seen that this implies that the rise of rate is much more rapid than the decline (*cf.* insert of actual curve in Fig. 3). The cycling is in all cases strongly damped and usually vanishes after the second or third division. What happens after the fourth or fifth division we have not followed.

In the cases where we see a clear cycling in the O_2 consumption it seems that the cytoplasmic cleavage is closely associated with an increase in the rate of oxygen consumption (shaded line in Fig. 7). The elevated rate may persist for 30 minutes to one hour after the division is through and has been termed an "excess consumption." It may reflect an oxidative debt left over from the cleavage, although this took place under perfectly aerobic conditions. Direct calorimetry has so far not yielded sufficiently detailed information to clarify this point. Rogers and Cole (1925) found a 10% drop in the rate of heat production at the first cleavage of eggs of *Arbacia punctulata*. Trurnit (1939) found that eggs of *Psammechinus miliaris* cleaved during a temperature maximum. His technique has been questioned (Zeuthen, 1946).

The earlier data on mass runs where cycling has been found appear on the whole to agree with our findings, in that most authors have observed cleavage to start at or near a low oxygen consumption (Fig. 7). Zeuthen (1949, 1950c) states for all his marine eggs that the cleavage furrow appears when the respiration is decreasing. His drawings actually show a relation more like that presented in our

Figure 7. By comparing his Figures 1 and 3 (1950c), the cell division in *Urechis* evidently did not correlate well with the cycling, but occurred as we have plotted it in Figure 7, one position seemingly contradicting the other. He also found periodicity in a frog egg that did not divide, and hence considered that the extra oxygen consumption was not closely connected with the cytoplasmic cleavage. We have observed two *S. purpuratus* eggs that did not divide and these did not show any change in O_2 consumption at the time when they should have normally divided.

INFLUENCE OF LOW OXYGEN TENSION UPON THE RESPIRATORY RATE AND CLEAVAGE

Experiments were made to find out to what extent lowering of the oxygen tension during an experiment would influence the rate of oxygen consumption. For this purpose the water was saturated with air instead of oxygen. Figure 8 shows that the oxygen consumption keeps on at normal rate until the last one or two minutes, when it abruptly stops. If the egg uses $180 \mu\mu\text{l}/\text{hour}$ or $3 \mu\mu\text{l}/\text{min.}$, and uses up all of the oxygen, then the consumption would not start to drop until there are only $6 \mu\mu\text{l}$ left. Six

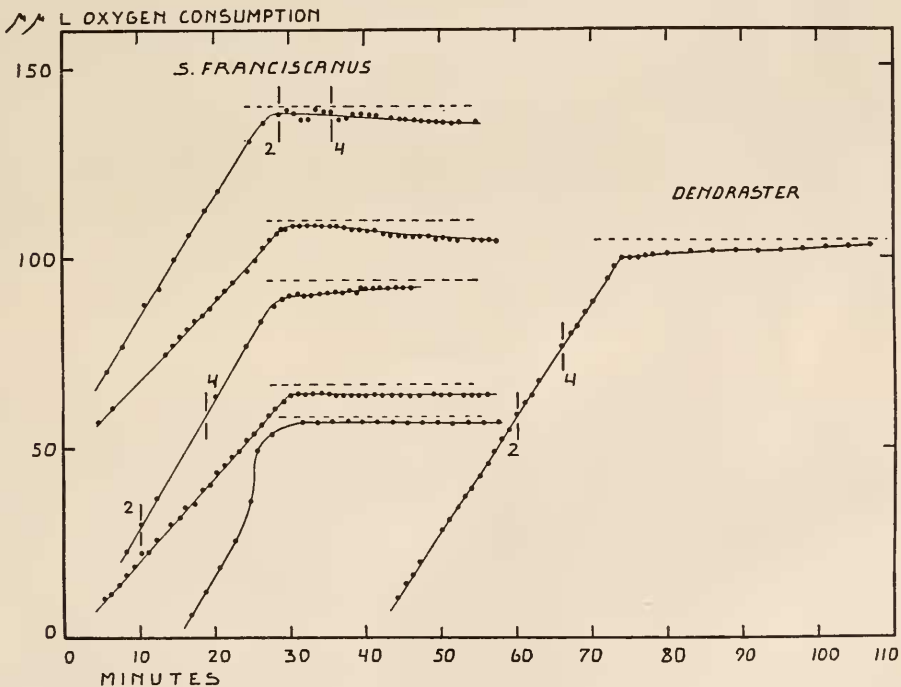


FIGURE 8. Oxygen consumption in single cells: experiments where the chamber water was charged with air instead of oxygen. At the sharp bend the cell has used up all of the oxygen. In the upper curve the oxygen ran out just as cell cleavage started. The cleavage continued without any oxygen consumption. The irregularities during this division are artifacts due to sticking of the pressure control, produced by the excitement of the operator. The position of each curve on the ordinate scale is arbitrary.

$\mu\mu\text{l}$ oxygen will exert a pressure of 1/100 atmosphere when dissolved in 20,000 $\mu\mu\text{l}$ water, and hence the oxygen consumption did not start to drop until a pressure of 7–8 mm. Hg was reached. This is in good agreement with Amberson's data on *Arbacia punctulata* (1928). In this species normal respiration keeps up until about 20 mm. O_2 tension is reached. The abrupt cessation of the oxygen consumption in our experiments is satisfactorily explained by the low O_2 capacity of the system compared to the relatively high rate of O_2 consumption.

Two of our curves showed a slight increase in flotation pressure after the cessation of oxygen consumption. This may be a sign of fixed acid formation with consequent release of CO_2 . Perlzweig and Barron (1928) found lactic acid formation in *Arbacia punctulata* and Runnström (1933b) and Borei (1934) found fixed acid formation at fertilization in two other echinoderms. In two other curves we found, however, a slight decrease in the flotation pressure.

In one instance (Fig. 8, upper curve) the cleavage furrow appeared during the very minute when the egg ran out of oxygen. The division went on to full completion, however, in spite of a complete cessation of the oxygen consumption. Evidently, therefore, cell division in this species can be completed without oxygen consumption and in spite of a presumably very low oxygen tension. E. B. Harvey (1927) showed that complete absence of oxygen would stop the mitosis in *Arbacia*, and likewise in *Echinus microtuberculatus* and *Strongylocentrotus lividus*. Amberson (1928) found *Arbacia* eggs unable to divide below an oxygen tension of 4 mm. Hg. *Arbacia punctulata* will continue cleavage after the addition of KCN (Blumenthal, 1930) and Örström (see Runnström, 1933a) found that *Paracentrotus lividus* would continue cleavage if brought into a pure nitrogen atmosphere at or after the diaster stage. Our observation may therefore be taken as a direct demonstration of what previous results have strongly indicated, namely, that cytoplasmic cleavage can proceed without oxygen consumption. The anaerobic energy may well be furnished by adenosine triphosphate, as Barnett (1951) found that this substance would produce cleavage in *Arbacia* eggs that had been stopped by anoxia.

CONCLUSIONS

The presented facts may be interpreted along the following lines. The cytoplasmic cleavage is associated with a relatively sudden transformation of energy. Since the cleavage, when started, will proceed anaerobically, the energy needed is not supplied directly by oxidation. As indicated by Barnett (1951) it may be provided by the breakdown of adenosine triphosphate. The increased oxygen uptake at or following the cleavage would then be indicative of a restoration of the ATP. Therefore the actual time interval of the extra energy demands of cleavage may be much shorter than the period of extra oxygen consumption. Since the conditions are at all times thoroughly aerobic, the rate of oxidative recovery would seem to be limited rather by the amount of oxidative enzymes present in the egg than by an inability to obtain adequate supplies of oxygen. It is possible that cycling may depend upon a certain excess of enzyme, not present in a non-cycling egg. It may also be that the excess oxygen consumption reflects directly the energy requirements of the mitotic nuclear processes that take place throughout this period before the next cleavage. Some of these processes may well be associated with an increased energy

exchange and could hence produce cycling of the oxygen consumption even if the cytoplasmic cleavage failed to occur, such as observed by Zeuthen (1946).

In our experiments the cycling is always strongly damped. It almost vanishes after the two or three first divisions. Zeuthen (1949) has found that later on cycling increases very markedly. Even if it can be demonstrated that this late cycling also occurs in single eggs, there will still be a minimum of cycling after the second or third division, and hence there can be no simple correlation between the wave amplitude and the steadily increasing number of cell divisions. It seems reasonable to believe that in later divisions the synchronism gets progressively poorer, and the exact nature of the late cyclings, therefore, needs further clarification. We must emphasize, however, that cell division in individual cells very often takes place without any demonstrable cycling. Quite regularly, therefore, the energy requirements for the different phases are apparently fitted nicely together within the limits of a steady constant flow of oxidative processes. It seems necessary to await much more detailed information before we can form a consistent picture of the energetics of cell division.

We wish to express our appreciation to the officers of the Milton Fund for making the above work possible, and to Dr. A. Baird Hastings we extend our sincere thanks for his unfailing interest and inspiration. We are indebted to Dr. Eric G. Ball for advice on the interpretation of the data. Dr. T. G. Thompson at the Friday Harbor Oceanographic Laboratories of the University of Washington supplied us with every possible assistance during our stay, as well as many helpful suggestions. We acknowledge with gratitude the cooperation of Dr. E. F. Swan, Dr. A. W. Martin and Dr. R. L. Fernald, from whose experience in marine biology we benefited greatly at Friday Harbor. Much credit is due to the efficient assistance of Mr. John Stevens in procuring equipment. We are much indebted to Dr. L. R. Blinks for providing us with excellent working facilities at Hopkins Marine Station, to Dr. A. C. Giese at the same station for invaluable assistance and advice on the handling of *Urechis*, and to Dr. C. B. Van Niel for inspiring discussion.

SUMMARY

1. In the present investigation we have analyzed the oxygen consumption during the first two to five cell divisions in single eggs from three echinoderms (*Strongylocentrotus purpuratus*, *S. franciscanus*, *Dendraster excentricus*) and from the echiuroid worm *Urechis caupo*, employing the reference diver technique (Scholander, Claff and Sveinsson, 1952a).

2. There is poor correlation between the cell volume and the oxygen consumption in individual eggs, both of which vary considerably.

3. The oxygen consumption during cell division may proceed without measurable change in rate (usual in *Urechis*, *S. franciscanus*) or it may show cycling correlated with the cell divisions (*Dendraster* and *S. purpuratus*). If cycling occurs, the cytoplasmic cleavage is associated with an abrupt rise in the oxygen consumption.

4. The cycling, if present, is strongly damped and often disappears after two to three divisions.

5. When started, cleavage can proceed without oxygen consumption (*S. franciscanus*).

6. Our runs on single eggs reflect in some respects the results gained by mass runs, but we are able to supply a more exact picture with respect to timing, curve shape, and variations in individual cells.

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